APPENDIX A

CLEANING SAMPLES FOR CD/CA

1) Prepare Samples
    a. Label 0.65 mL centrifuge tubes with sample ID–top and side
    b. Weigh samples ~0.5+mg
        i. Tare centrifuge tube and add samples with tweezers (stainless)
2) Precleaning
    a. Water Wash
        i. add ~250 μL mQ-water
        ii. ultrasonicate 20 minutes
        iii. resuspend
        iv. repeat until solution is clear
    b. Oxidizing Solution
        i. add 1 x 250 μL 50:50 mixture of 30% H_2O_2/2M NaOH
        ii. ultrasonicate 20 min
        iii. siphon off solution
        iv. resuspend 3 times with mQ-water
    c. Water Rinse
        i. add ~250 μL mQ-water
        ii. ultrasonicate 2 minutes
        iii. resuspend
    d. Methanol
        i. add 1 x 250 μL CH_3OH
        ii. ultrasonicate 5 minutes
        iii. resuspend 3 times with mQ water
    e. Water Rinse
    f. Bleach
        i. add 1 x 250 μL 50:50 mixture of 30% H_2O_2/1% $HClO_4$
        ii. wait 30–60 s
        iii. resuspend 2–3 times with mQ-water
    g. Water Rinse
    h. Transfer to acid leached polyethylene vials for cleaning
3) Cleaning
    a. Water Rinse–3 times
    b. Methanol Wash
        i. add 1 x 250 μL CH_3OH
        ii. ultrasonicate 2 min
        iii. resuspend 2–3 times with mQ-water
    c. Water Rinse

d. Nitric Acid Wash
    i. add 100 μL 0.2%HNO3
    ii. ultrasonicate 1 minute
    iii. quickly check pH (use 10 μL pipet)
    iv. resuspend 2–3 times with mQ-water
e. Water Rinse
f. Oxidizing Solution
    i. add 1 x 250 μL 50:50 mixture of 30% H_2O_2/0.1M NaOH
    ii. cover rack tightly
    iii. heat for 20 min in boiling water bath, ultrasonicate 5–10 s every 2 min (starting at 0), invert after each ultrasonication
    iv. Rinse 2–3 times with mQ-water
g. Water Rinse–2 times
h. Reducing Solution–ALL IN HOOD
    i. Make up working solution
        1. ~15 mL NH_3/citrate solution
        2. ~15 mL fresh NH4OH
        3. 1 x 1000 μL anhydrous hydrazine
    ii. Add 1 x 250 μL working solution
    iii. Heat for 30 min in boiling water bath, ultrasonicate 5–10 s every 2 min (starting at 0), invert after each ultrasonication
    iv. Resuspend 2–3 times with mQ-water
i. Water Rinse–2 times
j. Oxidizing Solution
k. Water Rinse–2 times
l. Nitric Acid Wash
m. Water Rinse–2 times

4) Transfer and Dissolve
    a. Transfer
        i. Add 1 x 50 μL mQ-water to each tube
        ii. Use 10 μL pipet with cut off pipet tip to transfer sample with a minimum of solution to a new labeled tube
    b. Postcleaning
        i. Add 1 x 100 μL 0.2% HNO_3
        ii. Leave for ~1 min
        iii. Aspirate
        iv. Resuspend 2–3 times with mQ-water
    c. Dissolve
        i. Add 200/250μL 2% HNO_3 and wait for sample to dissolve